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Research Article

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Antioxidant and Antibacterial Activity of Chitin, Chitosan and Shrimp Shells from Red Sea for Pharmaceutical Uses

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ABSTRACT

Background: The present study aimed to screen shrimp shell, chitin and chitosan methanolic extract to display potent anti-bacterial and antioxidant potency in vitro for some recently used antioxidants as food supplements and in the form of pharmaceutical dosages.
Materials and methods: shrimp shell, chitosan and chitin methanolic extract have biological potency as antiracial antioxidant. The tested materials were determined through disc diffusion technique against three strains Gram-negative bacteria (Escherichia coli; Klebsiella pneumoniae and Pseudomonas aeruginosa) and four Gram- positive bacteria (Bacillus subtilis; Micrococcus luteus and MERSA). Their antioxidant activity was investigated with DPPH (2,2-diphenyl-1-picrylhydrazyl) to measure radical scavenging potency and suppression of linoleic acid peroxidation.
Results: The chitin extract possesses a high potency against the tested organisms than the chitosan and shrimp shell. The values are based on the DPPH (41.0±1 µg/ml). Chitin also shows an increase in the radical scavenging properties.
Conclusion: Data of the present study stated that shrimp shell, chitin and chitosan act as an antioxidant agent because they might carry free radical scavenging and cytoprotective activity.

Keywords: *shrimp shell, chitin, chitosan, DPPH, Antioxidant*

INTRODUCTION

Both cellulose and Chitin are considered a highly rich natural biopolymer. The chemical formula of chitin is 2-acetamido-2-deoxy-b-d-glucose (NAG) monomers connected with β [1-4] linkages, which resembles the cellulose structure. Deacetylate (to variable degrees) formula of chitin is Chitosan where it dissolves in low pH (acidic) solutions. In most recent years, use of chinois products in nutrients and medications, besides processing aids, has taken extensive consideration as exotic artificial mixtures that are incapable of practical operation. In the previous few years, chitin and its deacetylate shape (chitosan) were paid special attention because they were applied broadly in the industries [1]. Nevertheless, only a little extent of care has been given to food industry of these multiuse bio products. Alteration of treatment rejects into appreciated products and alternate special constituents that are accepted as a suitable contest for food investigation and development attendant with several implementations of chitinous substance. Moreover, these biopolymers over an extensive assortment of exclusive implementation have bioconversion for making significant supplementary food products [2], protecting foods from microbial contamination [3-6], forming recyclable films [7-12], and cleansing water [13]. The chitosan possesses antibacterial property, which is mostly beneficial in the medicine field somewhere utilized for protection of medical equipment, for example, protective

gloves, dressings etc. Furthermore, it is utilized in the eradication of aquatic contaminated microorganisms in sewage and applied as natural nutrition protection and an outer surface covering various types of food. In current years, the antimicrobial property has been established beside diverse genus of microbes, which required extensive care especially with some strains of pathogenic microscopic organism [14-16].

The effects against microbial property of chitosan substance are significantly based on its material appearances, greatest particular molecular mass, and deacetylation status (DD). Chitosan that contains greater deacetylation degree has a larger ability as inhibitory property against bacterial strains [17]. Few years ago, many studies reported concerning chitosan that it could be used as bioproduct compound [15, 16]. Nevertheless, chitosan has great molecular weight, which affects reduced solubility and also becomes high viscosity solution, restricts its utilization as supplementary nutrition, foundation, farming and medicine manufacturing [18]. In addition, it is a bio product compound, nontoxic, glucosamine copolymer and N-acetyl glucosamine organized from chitin deacetylation that is considered as a chief component of the fish shell. Commercially, it is found in sewage of the sea food production industry [14, 16]. Chitin transforms to chitosan via the deacetylation processes at very strong alkaline environments by high temperature. The conversion of chitin to chitosan can be passed out successfully at slighter condition by enzymatic process to eliminate the acetyl groups from chitin construction [19]. Previous studies stated that chitin exoduses as a natural incomplete deacetylate form dependent on the source that is very hard to clearly differentiate between chitosan and chitin. Hence, the term chitin and chitosan can be utilized variably. Generally, when the gradation of deacetylation is higher than 50%, it is called chitosan [20]. The crab shell extracts of Liagorerubromaculata show antioxidant property and condense the free radical harms. The total antioxidant potential of fresh shelled crab shows concentrated antioxidant potential of 49% and minimum result of 32% is verified in rigid shelled crab. Soft shelled crab shows a decreasing influence during testing, and found that the most reducing capacity of 59% was observed in soft shelled crab. The lease reducing capability of 42% was documented in solid shelled crab [21]. DPPH is also reflected as a respectable moving model for peroxyl radicals. Antioxdant property is an important characteristic and greatly significant for natural life. Numerous biological roles of antioxidants are documented like anti-mutagenicity, anti-carcinogenicity and anti-aging among others. Previous reports suggested that protein from L. rubromaculata crab and Hemolymph showed DPPH scavenging activity. The influence of antioxidants on DPPH radical scavenging was due to their hydrogen giving facility [22]. Latest results on chitosan polymerization take tense significant consideration as the acquired substances are more soluble in water. The favorable advantages of chitosan and its oligosaccharides enhance its uses in the therapy as anticancer [23] neuroprotective [24] against microorganisms and fungi [25,26] and anti-inflammatory [27].

MATERIAL AND METHODS

1.1. Samples:

Samples of chitosan and chitin were collected during May 2014 from Dammam city in East region of Saudi Arabia. Shrimp shells were collected from the fish market in Dammam city. All shells were from a single species. The chitosan and chitin were collected from factory.

2.2. Samples preparation

By using distilled water, the shrimp shells were cleaned to remove debris and other impurities. Formerly boiled samples of shrimp shells for the purposes of removing wastes for one hour, after that putting the shells to dry at high temperature (160 °C) for two hours in an oven to create them very hard and also to clear up the crystal clear construction of samples [28], were carried out. Finally, the dried shells were turned to fine powder by ground in a machine for grinding. Samples were then maintained in sterilized bags in laboratory until used. Chitosan and chitin were obtained from factory.

2.3. Extract preparation

A quantity of 10 g of dried samples was immersed in 100 ml of methanol overnight. The remained extract was collected by separation, vaporized below reduced pressure on 40°C up to dryness; then, diluted by dimethyl sulfoxide (DMSO) and kept on 20°C [29].

3.3 Determination of antioxidant

The firm free radical molecules DPPH (1,1-diphenyl-2-picrylhydrazyl) in substantial scavenging experience was passed over by making use of spectrophotometer 30. Then, (50 µl) of experimented samples was added to a volume of 5 ml from DPPH soluble in ethanol at concentration 0.004%. Then tested extracts were kept warm for 30 min.

Absorbance were deliberate at 517 nm wavelength versus blank using spectrophotometer. The proportion (%) of DPPH radical scavenge is calculated using the equation below.

$$1\% = (1- AS/ AC) \times 100 \quad (1)$$

Wherever AC is the controller reaction absorbance having wholly components not including the verified extract and AS means verified extract absorbance. An inhibition percentage (%) was established from plotting graph beside samples concentration. Wholly tests remained done in replica.

Test bacterial strains

Seven strains of microorganisms were got from King Abdul Aziz Clinic, Jeddah city, Saudi Arabia**,** they were: Escherichia coli; Klebsiella pneumoniae; Pseudomonas aeruginosa; Bacillus subtilis; Methicillin-Resistant Staphylococcus aureus; Staphylococcus aureus and Micrococcus lutes). Strains of microorganisms were delivered by Microbiologics® USA. The test microorganisms were cultured on special culture media (nutrient agar slants). Incubation was at 37°c. The agar slants were maintained at 4°C.

2. 4. Antibacterial activity

The antibacterial action was determined by means of [31]. By using DMSO as –ve control, samples of microorganisms' strains were prepared in replication. Then, tested bacterial strains were incubated. Finally, antibacterial action was detected by measuring the inhibition area [32].

5. Statistical analysis

Every test had three replications and three calculations were performed. The averages of variables and standard deviation were recorded. Student t- test was done to detect any significant variations among the control and tested samples.

RESULTS AND DISCUSSION

Adding active antioxidant substances is essential to prevent infections associated with increase in the release of free radicals. These free radicals have great influence on different organs in the body, which leads to circulatory system disorder, lipid peroxidation or atherosclerosis. A broad variety of antioxidant action of biopolymers is unlimited [1]. Many studies were carried out on plant seeds to explore their antioxidant activity, in the present work, the extracts of three biopolymers were tested to explore the antioxidant activity by using the known test DPPH radical scavenging activity, and as antibacterial activity.

DPPH (Radical Scavenging Assay)

DPPH substance is a stable organic free radical with violet color, which provides consumption extreme within 515 to 528 nm. Active receipt proton from hydrogen giver, mostly from phenolic compound, and misses has chromophore and converted to yellow. This assay depends on determination of phenolic compounds concentration, the rises in hydroxylation of the phenolic materials are associated with increase in the level of DPPH radical scavenging and therefore, the antioxidant potency of a plant extract or a related compound similarly rises [33]. Depending on the amount of phenolic constituents in the tested substances, the DPPH radical scavenging power of the plant extract is relatively described [34].

DPPH assay can be used for determination of the hydrogen donating capacity of diverse extracts. The combination of samples and DPPH reagents assay incubated at 370C and kept at this condition for 30 minutes before the DPPH assay. Then, it was detected that all the extracts influenced temperate to great free radical scavenging activity. Tested samples revealed an inhibition. In Figure [1], DPPH assay shows the antioxidative probability of hydrolytic product. Chitin revealed a potent antioxidant efficiency (29.6%). While, chitosan showed a low rate of inhibition, which reached 20.8%; whereas, the antioxidant activity of shrimp shells reached 23%.

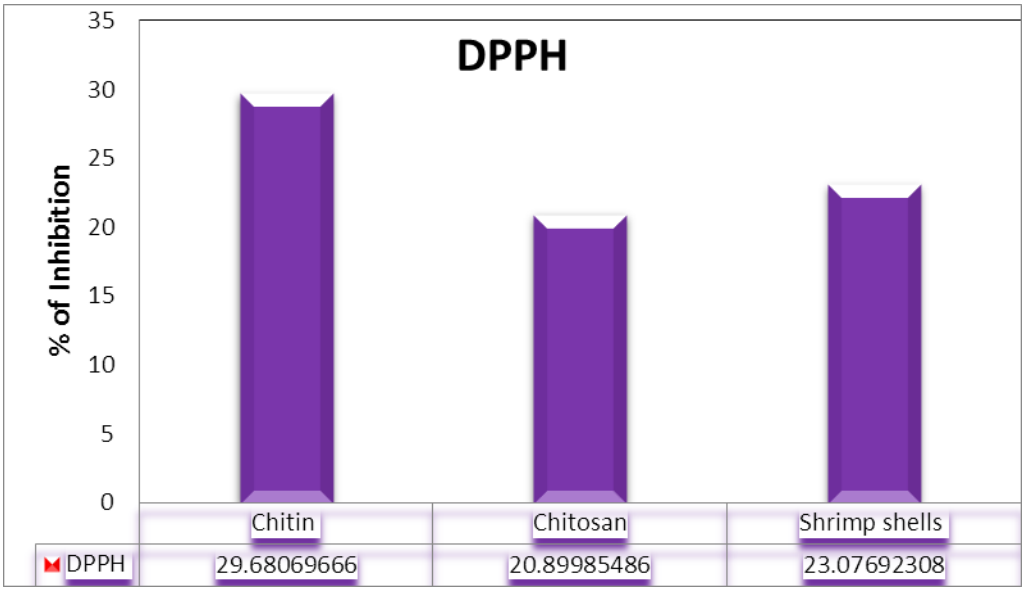

Fig (1): (DPPH) radical-scavenging activity of Shrimp, chitosan and chitin

The results revealed that the ethanolic extracts of the three samples possess a high antioxidant activity in agreement with [18] results, which examined higher extract yields from chitin and chitosan, then stated that chitosan scavenging activity attributable to response through the free radicals and backward the free amino groups to fixed macromolecule proceedings (the amino group with radicals) can transact ammonium groups by taking hydrogen ions from the solution and reacting with radicals through another reaction. The scavenging activity of chitins and chitosans improved with excess concentration ranged from 1 to 2% (w/v). The effects showed that the radical-scavenging activities of Squalled chitin was unaffected by the diverse applications. Furthermore, this limitation differs inside species. In 2013, [35] suggested that the antioxidant activity of extracted chitosan is required diverse kinds of chitosan currently presented in the marketplace, which must be advanced towards more additional to similar requisite standards. Using design for medicine delivery, different production techniques are consumed to make drug of chitosan cells transferors by concerning on chitosan cells parameters as fractious linker application, chitosan particles mass and treating environments totally as affecting relief degree of the full medicine. Chitosan appears to be the biopolymer for the improvement of novel products, for example, soluble products of chitosan, Quaternarized derivatives, Caboxyalkylation, sulfonated derivatives of chitosan, Chitosan esters, and N-trimethylene chloride chitosan are conjugated [35].

Moreover, there are many reports indicated that chitosan, chitin, and peptides have anti-oxidative [36] and anti-carcinogenic activity [37] with the purpose of increasing the usage of these chitin/protein in the wastes of shrimp shell incubated Serratia sp. TKU017 in the maximum culture environment for 1-5 days. The skill of DPPH scavenging was the tested antioxidant action. The date signified that TKU017 culture supernatant (2% SSP) incubated for 4 days had the highest antioxidant action, wherever the DPPH scavenging facility of the TKU017 culture supernatant was about 70%. Additionally, examinations as an end result of different carbon/nitrogen (SPP) on the making of antioxidant materials by TKU017, the antioxidant actions were 15-20% in the SSP and 20-30% in the SPP supernatants. Furthermore, it was established that the antioxidant action developed after fermentation by TKU017. SSP was the suitable carbon/nitrogen source intended for antioxidant materials manufactured by strain TKU017. It is estimated that even though the treatment (121oC for 15 min) brokedown the marine waste and yields some of the anti-oxidant materials, the greatest parts of the anti-oxidant materials are prepared by strain TKU017.

3.1. Antibacterial activity

The data of table [1] show the results of the antibacterial influence of shrimp shell, chitosan, and chitin methanolic extract, which are resolved by anti-microbial resistance test methods beside wholly microorganisms' strains e.g. B.subtilis, MRSA, S.aureus, E.coli, K. pneumonia and P. aeruginosa at sample extracts concentrations 200 mg/ml, chitin extracts presented greater effect beside the verified strains than chitosan and shrimp shell. The antibacterial influence of shrimp shell, chitosan and chitin methanolic extracts were seemed to alter actually in efficiency, then bacterial strains revealed greatly unaffected and some strains of tested bacteria further affected by methanol extracts

of shrimp shell, chitosan and chitin. It was observed that the chitin methanolic extract presented big diameter zones of inhibition beside E. coli, P. aeruginosa, S. aureus, K.pneumonia, B.subtilis and MRSA with inhibition zones 33.33mm, 23.33mm, 22mm, 20mm, 18mm and 16mm, correspondingly (Table 1). The shrimp shell methanolic extract showed moderate activity beside wholly strains tested. The biggest inhibition zones made by shrimp shell methanol extract were beside E. coli with inhibition area 30mm. Moreover, shrimp shell methanol extract had no activity against S. aureus, while the chitosan methanolic extract presented a lowest effectiveness against the tested bacteria associated to those got with other extracts. Antimicrobial activities of regular positive antibiotic controller (streptomycin) presented an inhibitory influence on all verified bacteria. These findings were in accordance with [38] who recommended that there is a continuous and critical necessity to determine recent anti-microbial materials with various chemical composition and novel mechanism of action as a worrying increase in the occurrence of new and re-evolving infectious diseases. [39] described that the Chitin displayed a bacteriostatic influence on some strains of microorganisms like Escherichia coli ATCC 25922, Bacteroides fragilis, Vibrio cholerae and Shigella dysenteriae; while, chitosan revealed a bacteriostatic action on most strains of microorganisms, excluding Salmonella typhimurium. In addition, two polysaccharides such as chitosan and chitin seemed to be bacteriostatic rather than bactericidal activity on different strains of bacteria. Later, the mechanism of action as antibacterial of the two polysaccharides creates the bacteria alternation and therefore kills bacteria possibly during starvation of nutrient materials and oxygen requirement.

The antibacterial action of chitosan is greater than that of chitin, all of them inhibit the growth of cultured microorganisms, and this is referred to that chitosan is rich with polyatomic amines, which can connect with the negatively charged remains of proteins carbohydrates and lipids found on the cell surface of Gram-negative bacteria strains [40].

Table1: Antibacterial activity of chitin, chitosan and shrimp shell extracts against some pathogenic bacteria

Mean diameter of inhibition ±Standard error mean (SEM)						
Extract used	**B.subtilis**	**MRSA**	**S.aureus**	**K.pneumoniae**	**E.coli**	**P.aeroginosa**
Chitin	20.00±0.00	16.00±0.00	18.00±0.58	22.00±1.00	33.33±1.53	**23.33±1.15**
Chitosan	13.00±1.00	00.00±0.00	13.00±1.00	15,33±0.58	14.67±0.58	**16.67**±1.53
Shrimp shell	15.00±0.00	15.00±0.00	00.00±0.00	17.00±0.00	30.00±0.00	**18**.00±1.00
Streptomycin	25.00±00.00	23.67±0.33	19.00±00.00	25.00±00.00	23.00±00.00	22.00±00.00

This is coordinated with findings of [41] who examined the efficiency of chitosan derived from shrimp with different concentrations as inhibitor of some strains of microorganisms as Escherichia coli [42]. The statement of using scanning electron microscopy (confocal laser) defined that chitosan oligomers are occurred inside E. coli capable to pass over bacterial cell membrane, also it produces escape of glucose and lactate dehydrogenase substance from E. coli cells. These records provide the action mechanism of chitosan material, which can inhibit the growth of different strains of bacteria cause a cross-linkage cover among the polycations of chitosan and the anions on the bacterial surface that alternate the membrane permeability [41,43]. In addition, the growth-inhibitory influence of most bacterial strains reported when 50mg/ml of methanolic extract given. Another trial was carried out by [44] who showed the way of chitosan operation for treatments of infections with Staphylococcus simulans [22] and S. aureus SG5 [11]. Chitosan was found to be a dose-dependent, which needs the growth-inhibitory influence for coincident binding of the cell membrane to minor cellular constituents. Furthermore, [45] established that excellent anti-bacterial efficiency of chitosan derived from original source beside Gram-negative (Salmonella Paratyphi) and Gram-positive strain (Staphylococcus aureus). Chitosan might be an important substance of medicines, which could be beside infection of bacteria.

CONCLUSION

It is concluded that chitosan and chitin are derived from natural sources, which have many advantages such as low-cost, abundant useful and safe constituents. The current study similarly presented that numerous drugs could be

produced extracting from chitosan and chitin substance that have great antimicrobial action. Also, it showed the probability of developing chitosan as an effective substance for bacteria inhibition.

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